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An Evaluation of X-Radiography Studies in Estimation of Gastric Emptying Time (GET) in Whiting (*Merlangius merlangus* L.)

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Abstract: This study investigates the use of X-ray technique in estimating the gastric emptying time in whiting (*Merlangius merlangus* L.) fed in the laboratory conditions. All the experimental fish was fed to satiation with their natural prey sprats. The disperse type radio-opaque compound Barium Sulphate ($BaSO_4$) paste was pre-injected in the body cavity of sprats in proportion to the body weight (0.1 to 0.15 ml/g sprat) prior to the experimental feeding. The $BaSO_4$ paste was found suitable to portray the actual movement of food/prey items over different time since feeding that for fish that took larger satiation meal required longer time to empty the stomach. Results of this study disapproved the earlier claimed that the gastric emptying times (GET) remained constant as meal size increased. Results on modeling of satiation feeding were also discussed.

Key words: X-radiographic, satiation feeding, radio opaque markers, gastric emptying time

Introduction

Production in both wild and cultivated fish populations are largely depends on food consumption and the way in which food is utilized within the body. Important aspects of trophic dynamics of fish include appetite, meal size and frequency, rate of gastric evacuation and assimilation efficiency and many other methods have been devised to study these parameters. One of the popular methods is to use the X-radiographic techniques to observe and describe the movement of food items in the alimentary tracts of fish after feeding. Yet, this method has been subjected to serious debate due to ambiguity on the susceptibility of the various types of radio-opaque markers to various species of the experimental fish. The key point in such a study is to ensure and validate that the types of radio opaque used are well mixed, move and representing the emptying of the food item as it passes along the alimentary tracts. Particulate markers such as ballotini (lead glass beads), electrolytic or metallic iron powder (filings) and lead >shot= have been used. Iron particles worked well in feeding studies of the *Atlantic salmon* (*Salmo salar*, Talbot and Higgins, 1983) but not in similar studies for *Arctic charr* (*Salvelinus alpinus*, Jorgensen and Jobling, 1988). Ballotini have been widely used to estimate GET and gastric emptying rate (GER), Hossain *et al.* (1998) used them in an X-ray study of African catfish fingerlings and found that they did not affect feed preference and gastric emptying rate. Sims *et al.* (1996) successfully estimated the rates of gastric emptying and return of appetite using formulated diet containing radio-opaque glass beads in lesser spotted dogfish (*Scyliorlhinus caniculus*). In contrast, several studies carried out to test the validity of particulate markers indicated that there were significant effects depending on particle size and density, on estimates of GER and GET caused by retention of the marker (Talbot and Higgins, 1983; dos Santos and Jobling, 1990; Jobling *et al.*, 1995a,b).

Seyhan *et al.* (1998) studied the gastric emptying of natural food (*Sprattus*) by whiting (40-400g bw) at range 8 to 16EC and reported that gastric emptying of meals up to 4% of body weight was usually complete within 50 h., independent of meal size. In contrast, Bromley (1988) found much longer gastric emptying times (GET) up to 100 h. - under similar conditions when his fish consumed voluntary meals, which were up to five-fold, larger.

To resolve this disagreement, an X-radiography technique was carried out using larger meal sizes (voluntary satiation feeding). The work was carried out using the radio-opaque marker, barium sulphate paste ($BaSO_4$), injected into whole sprat to act as a contrast medium. The marker was held back relative to the food in Arctic charr *Salvelinus alpinus* (Jorgensen and Jobling, 1988), and in Atlantic cod, *Gadus morhua* (dos Santos and Jobling, 1991). But Seyhan (1994, unpub. observ.) found that the injected marker remained mixed with the food and the relationship between stomach volume (SV, ml) and body weight (W) for whiting (48 to 695 g, n = 42) was:

$$SV = 0.067\ (SE,\ 0.0027)\ W \quad \text{---------------------------------} \quad (1)$$

which may be used as a useful predictor of satiation meal size for natural food items.

Materials and Methods

The experimental fish: Whiting (35B550g body weight) were captured live, from the nearby Menai Strait using hook and line or fish trap, or by trawl net using the R/V APrince Madog@ in the coastal waters mainly east of Anglesey (mainly 53E18=-53E26=N; 3E45=-4E15=W) during Autumn (October B December, 1998). They were transported to the Fish Laboratory; Menai Bridge where they were acclimatized at ambient temperature (10 ± 2°C) in 4000L aerated holding tanks for at least 4 weeks prior to the start of experiments.

Plate A: Close up view of partially frozen fresh whiting alimentary tract showing details of the sections containing whole sprats mixed with radio opaque $BaSO_4$.

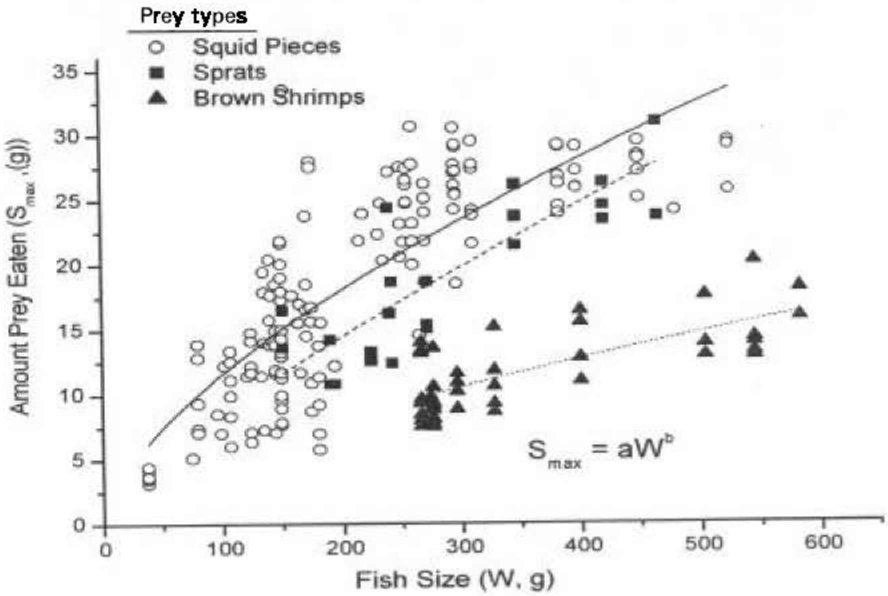

Fig. 1: Relation between size of satiation and meal (S_{max} g wet wt.) and fish size (W,g wet wt) of whiting feeding on different prey types (temperature range 9.7-19.3°C).

Table 1: Comparison of allometric parameters a and b to indic ate the variation of satiation meal S_{max} of different prey types eaten by whiting of various sizes S_{max} = a*W^b). (Temp. range: 9.7-19.3°C)

Prey (meal) types	Estimated parameters		R	n (df)
	a±SE	b±SE		
Squid pieces	0.608±0.143	0.640±0.042	0.814***	147(145)
Sprats	0.389±0.212	0.696±0.095	0.790***	24(22)
Brown shrimp	0.57±0.147	0.651±0.096	0.726***	41(39)

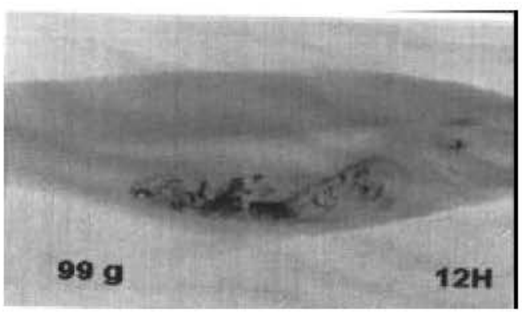

Plate. B: An X-ray images of 99g fish stomach ingesting 10.4g labelled food taken after 12h since feeding.

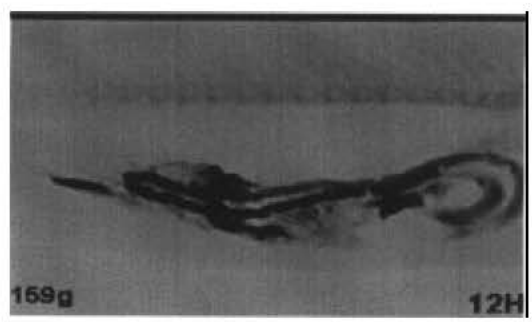

Plate. D: An X-ray image of 159g fish stomach ingesting 7g labelled food taken after 12h since feeding

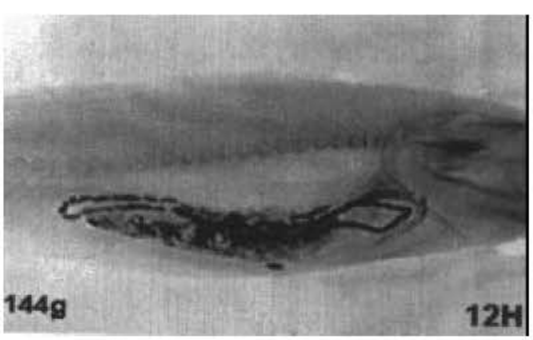

Plate. C: An X-ray image of 144g fish stomach ingesting 10.9g labedlled food taken after 12h since feeding

Plate. E: An X-ray image of 211g fish stomach ingesting 14.6g labelled food taken after 12h since feeding

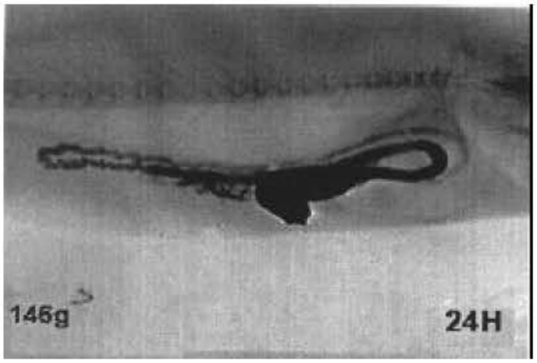

Plate. F: An X-ray image of 146g fish stomach ingesting 9g labelled food taken after 24h since feeding

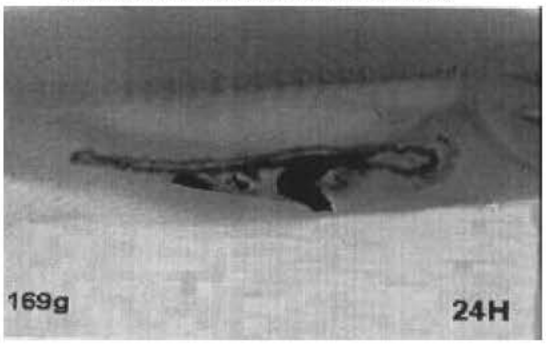

Plate. G: An X-ray image of 169g fish stomach ingesting 6g labelled food taken after 24h since feeding

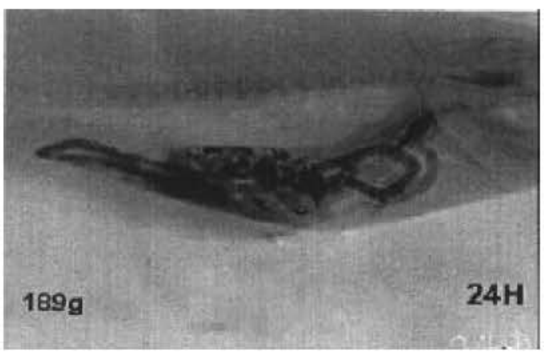

Plate. H: An X-ray image of 189g fish stomach ingesting 8.5g labelled food taken after 24h since feeding

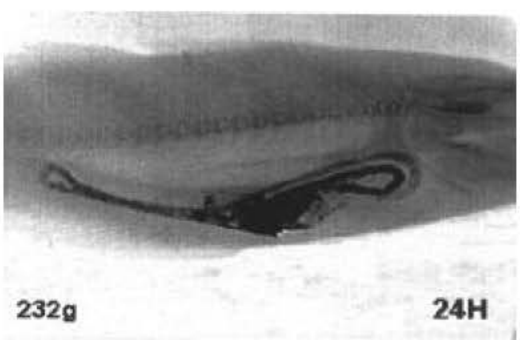

Plate. I: An X-ray image of 232g fish stomach ingesting 11.2g labelled food taken after 24h since feeding

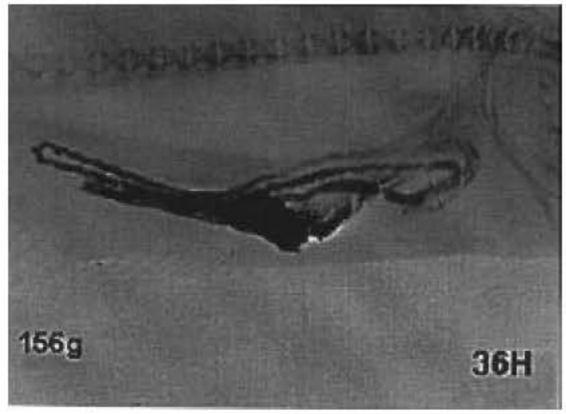

Plate. J: An X-ray image of 156g fish stomach ingesting 16g labelled food taken after 36h since feeding

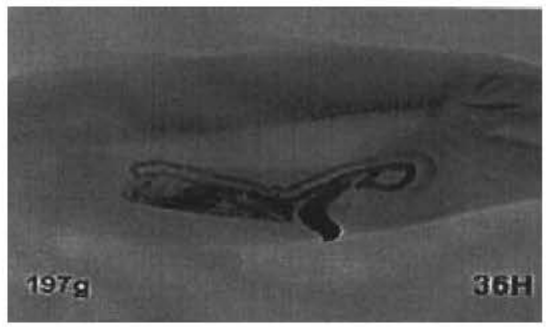

Plate. K: An X-ray image of 197g fish stomach ingesting 16g labelled food taken after 36h since feeding

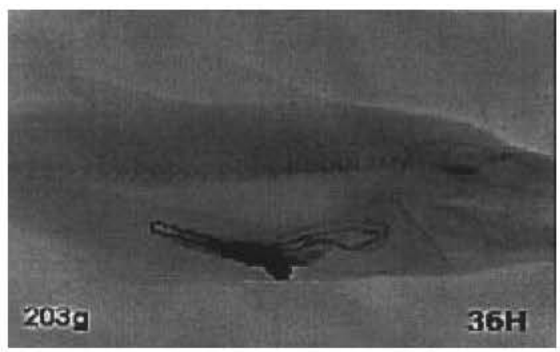

Plate. L: An X-ray image of 203g fish stomach ingesting 6.1g labelled food taken after 36h since feeding

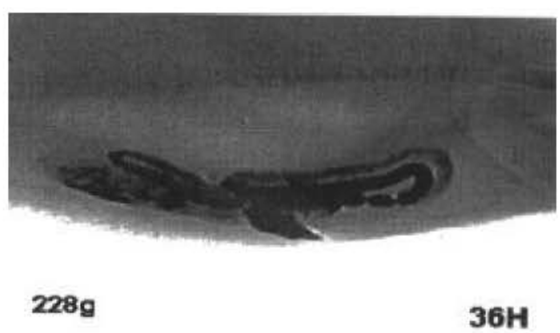

Plate. M: An X-ray image of 228g fish stomach ingesting 17g labelled food taken after 24h since feeding

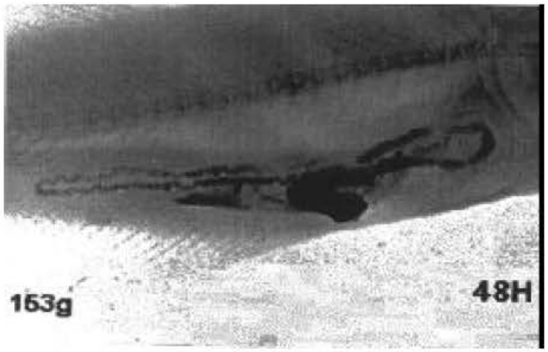

Plate. N: An X-ray image of 153g fish stomach ingesting 9.6g labelled food taken after 48h since feeding

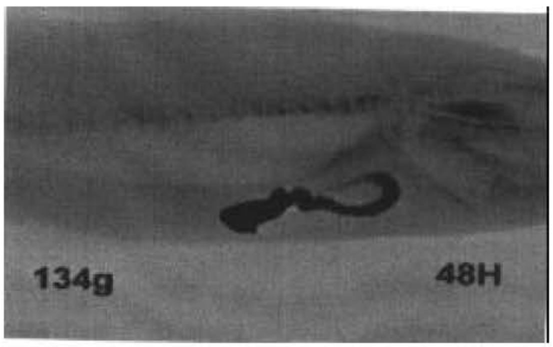

Plate. O: An X-ray image of 134g fish stomach ingesting 4.3g labelled food taken after 48h since feeding

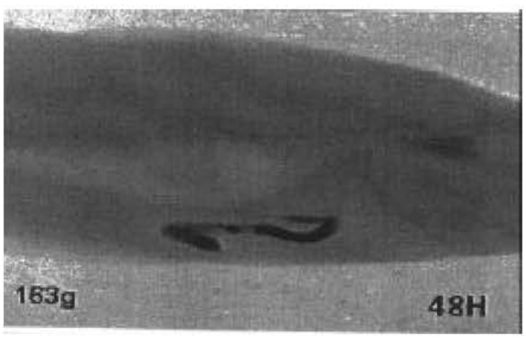

Plate. P: An X-ray image of 163g fish stomach ingesting 4.9g labelled food taken after 48h since feeding

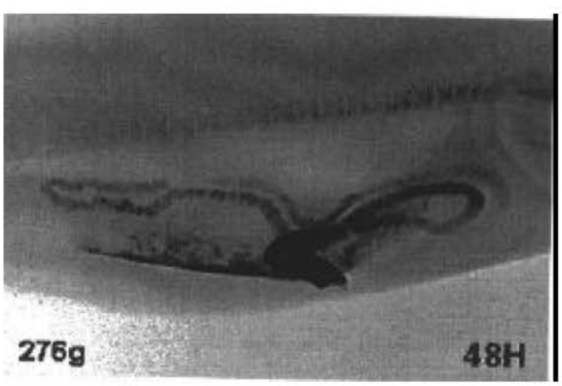

Plate. Q: An X-ray image of 276g fish stomach ingesting 27.6g labelled food taken after 48h since feeding

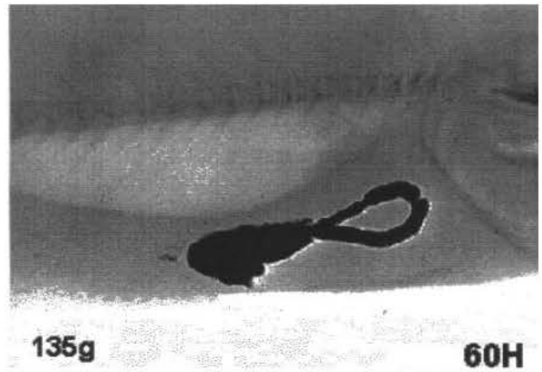

Plate. R: An X-ray image of 135g fish stomach ingesting 5.4g labelled food taken after 60h since feeding

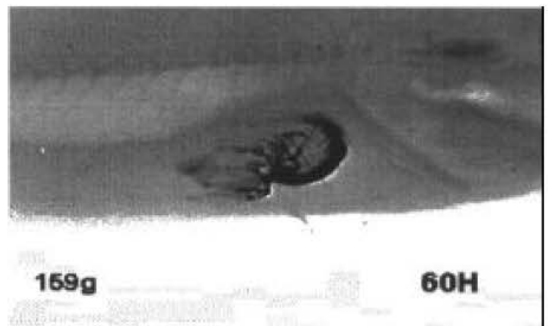

Plate. S: An X-ray image of 159g fish stomach ingesting 4.8g labelled food taken after 60h since feeding

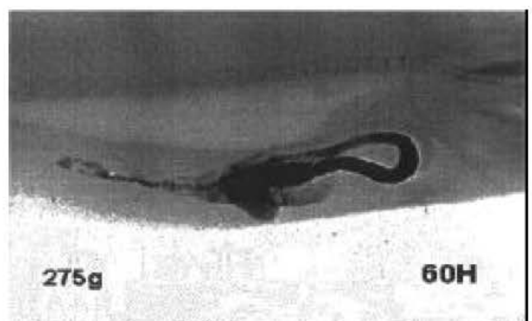

Plate. T: An X-ray image of 275g fish stomach ingesting 8.3g labelled food taken after 60h since feeding

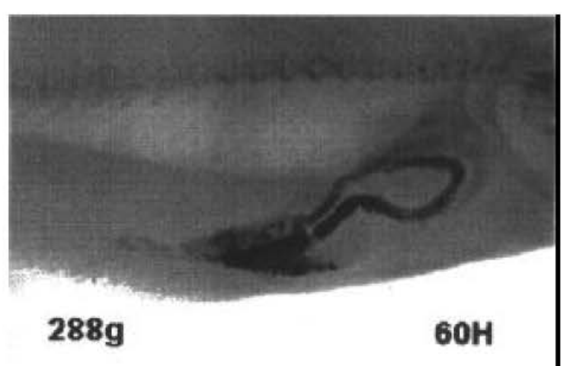

Plate. U: An X-ray image of 288g fish stomach ingesting 25g labelled food taken after 60h since feeding

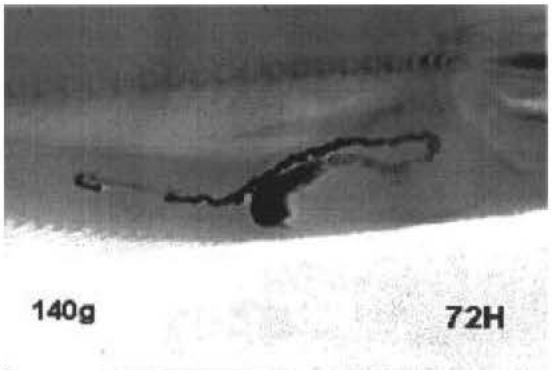

Plate. V: An X-ray image of 140g fish stomach ingesting 13.2g labelled food taken after 72h since feeding

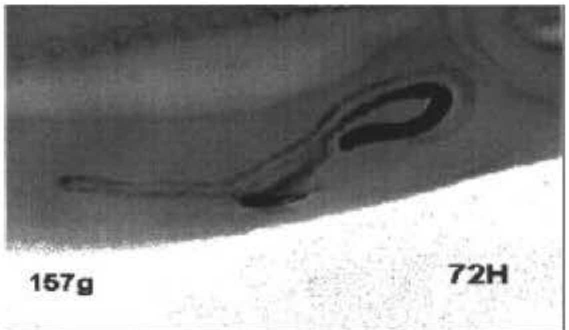

Plate. W: An X-ray image of 157g fish stomach ingesting 13.2g labelled food taken after 72h since feeding

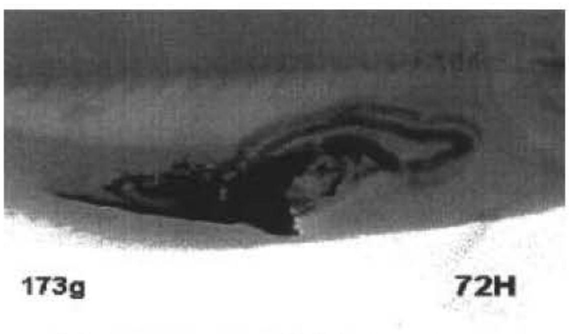

Plate. X: An X-ray image of 173g fish stomach ingesting 18.6g labelled food taken after 72h since feeding

Plate. Y: An X-ray image of 183g fish stomach ingesting 18.9g labelled food taken after 72h since feeding

Before each experiment, appropriate numbers of healthy fish were transferred into 250L raceway tanks and acclimatized for 14 days with test meal (fresh sprats) prior to the start of the experiment.

Preparation of radio opaque meals: X-radiography was used to observe images of food in the alimentary canal of fish at stated times after feeding. A small amount of barium sulphate paste (Ca 0.1-0.15 ml) was injected into the dorsal muscles of whiting common prey, fresh whole sprats using a hypodermic syringe (1ml). It is important to use amounts of barium sulphate, in each sprat, which are proportional to its weight to produce similar image densities as the food breaks up. The relationship between the amount of barium sulphate paste that could easily be injected (Fig. 1)and the sprat's body wet weight (Prey size, g); each 1 g of sprat contained 0.110 ± 0.002 ml barium sulphate paste ($r = 0.8856$; $N = 40$; $P < 0.0001$).
The sprats (containing radio opaque marker) were kept frozen prior to start the experiment. All experimental fish were deprived of food for 96h and then offered pre-weighed labelled sprats to satiation. The exact amount eaten was recorded for each fish. A serial slaughter sampling at different time since feeding was adopted in this experiment. In a serial slaughter sampling, at least five fish at each selected time were killed by stunning and destroying the brain. Each fish was X-rayed and the films immediately processed in the adjacent dark room.

The X-ray technique and the film processing protocol : A portable X-Ray machine was used. The objects (i.e. live or dead fish) were placed on a cassette (Agfa) containing a screen (Curix Blue C2) and film (Curix RP1 Plus 100 NIF or Curix Blue HC-S Plus 100 NIF, 18 x 24 cm) 30-31cm from the X-ray source. Exposure times of 0.2-0.3 second produced satisfactory images of food and indigestible solids in the stomach and intestine of the whiting.
The X-ray positive images were captured digitally using a Digital video Camera (Sony Hi8XR) and later downloaded into a Personal Computer. The images were further enhances using Paint Shop Pro 6.0 Software.

Feeding experiment: The aim of this experiment was to estimate the maximum feeding capacity of whiting (S_{max}) fed on test diets, squid pieces, whole sprats and whole brown shrimps. After a week in the holding tank, whiting (35-500 g) were individually transferred into 250L tanks. They were acclimatized for 7 days with intermittent feeding to satiety with squid pieces, (0.8 to 1.8g), sprats (0.7-2g) or brown shrimps (0.8-3.5g). Sufficient amounts of food items were weighed and test meals offered to the fish for about 15-20 minutes until the fish showed signs of satiety. Uneaten items were recovered, weighed and amounts of food ingested by each fish calculated. Test were repeated every 96h over the following days until satiation amount stabilized. Since no temperature control available, the temperature during the course of the experiment (6 months) varied between 9.7-19.3°C.

Results

X-radiographic observation: The large ellipsoid stomach of whiting can be easily distinguished from the mass of pyloric caeca, intestine and rectum after dissection (Plate A) and in the X-ray films. The stomach is separated from the intestine by the pyloric sphincter that is encircled by the pyloric caeca, and is followed by a narrow, thin-walled intestine leading to an expanded rectum (Plate A). The alimentary tract contained digested sprats and the associated $BaSO_4$ paste; comparison of stomach contents and X-ray images showed the marker component ($BaSO_4$) moved along with the test meal digesta and showed similar gastric emptying times. When filled with digesta, the intestinal wall expanded forming a very delicate thin walled tube. Plates B - E showed the X-ray image of small and medium sized whiting 12h after ingesting meals of 8-10% of their body weight (8 to 19 g) of labelled sprats. The stomachs are full, food has already entered the proximal and distal arms of the intestine loop but has not reached the rectum.

In Plates D and especially E, labelled chyme can be seen within parts of the pyloric caeca. Plates F B I are images taken 24h after feeding. Labelled material has reached the rectum in smaller fish (Plates F and G) but food is still present in the stomach (see Plate H). Dissection showed that 35-55% of the original meal was still present at this location in these fish. One fish (189g) ate approximately 75% (9g; Ca 5% of body weight) of its estimated maximum capacity (13g). Plate J B N show images after 36h. Stomachs were only partially empty (70 B 80% of the meal had moved on) and the labelled digesta fully filled the intestinal tracts with most of it concentrated in the rectal region. The smallest fish in the group (156g) ingested 16g of fresh whole sprats comprised more than 100% of the predicted maximum capacity of stomach (11g) from Seyhan's formula (i.e. equation 1).

After 36h only 31% (5g) of the meal was left in the stomach. Similar excessive ingestion was also displayed by the fish in Plate K (197g), which consumed 16g of sprats leaving 5.4g (34%) in the stomach after 36h. Larger fish (Plates L, M) retained 20-30% of the original meal at this time.

Plates N - Q show that two fish had emptied their stomach within 48h as expected from Seyhan's study. These fish (134 and 163g) had ingested only 4 and 5g of sprats respectively and are comparable with the previous experiments by Seyhan (1994, unpub. observ.). However, the whiting in Plates N and Q had not fully emptied their stomach; 23 - 25% of the meal remained. These whiting accepted large meals - Ca 6 - 10% on their body weight - and required more than 48h to completely empty their stomach. Plates R - U showed that there were still some digesta left in the stomach of several fish even after 60h. Those fish with empty stomachs had ingested around 30-45% of their expected maximum stomach capacities, only Ca 3% of their body weight. The larger fish in Plate U (288g) ingested 25g of sprats (9% bw) and still retained 3g in the stomach after 60h. The fish that took small meals had digesta concentrated mostly in the posterior intestine and rectal regions. Those taking large meals had digesta in all sections of the alimentary. Interestingly, Plate S showed a 159g fish that ingested around 5g of sprats (3% of body weight), which had completely emptied the stomach, defecated all the digesta, but left residues of label in the pyloric caeca. Plates V-Y show the status of digesta in the alimentary tracts after 72h. Small fish (140 and 157g) that had ingested more than 100% of their estimated maximum stomach capacity (13.2 g, 7 - 9% bw) had apparently emptied their stomach, leaving most of the digesta concentrated in the posterior intestine and rectal region. Similar fish (173 and 183g) that ingested more than 10% of their body weight (19g) or 158% of the estimated maximum stomach capacities, retained small amount of digesta in the stomach (4 - 5g, 20 - 25% of the total amount ingested). Whiting which consumed more than 10% bw meals had gastric emptying times greater than 72h.

Modeling of satiation feeding: An initial modeling of satiation feeding is important to estimate the feed intake capability in fish of various sizes (Table 1 and Fig. 1). For whiting of a given size, the largest weight and volume ingested occurs when they are fed on squid pieces, followed closely by sprats whilst ingestion of brown shrimps was lower. Satiation amount (S_{max}) increased allometrically with fish weight (W) as; $S_{max} = aW^b$. where b = 0.64 - 0.7. The variations in "a" indicated that different packing factors do exist for different prey species when fed to whiting. A whiting of 500g, which Seyhan considered should have a stomach volume of 33.5 ml, ingested 32.5g of squid, 29.4g of sprat but only 14.7g of brown shrimp. However, smaller whiting ate larger meals than predicted from Seyhan's model; a 50g fish typically eats 7g of squid but has a predicted stomach volume of only half this size. So, that the stomach increase in volume allometrically with fish size rather than the linear relationship suggested by Seyhan. Therefore his original data set can be re-described by fitting a power equation but fixing the average value of b = 0.62 (found from the present study) as; Stomach volume $= 0.438W^{0.662}$ and the 95% confidence limits of the fit reduce to 31%. Whiting of 50g bw would have on average a stomach volume of 6 ± 1.8ml and 500g fish 27 ± 8.1 ml.

Discussion

In this study, the disappearance from the X-ray images of the whiting of the radio-opaque marker ($BaSO_4$) injected into the prey closely followed the change in stomach contents obtained by dissection. This indicates that this technique is useful as a tool to observe both food intake and the gastric emptying process in the whiting. However, the use of barium sulphate paste within the natural food may itself affect the digestion rate of the item. Great care is required during injection of the sprat to retain the measured amount of paste in the sprat body cavity due to its delicate tissues. The problem was minimized by injecting an amount in proportion to the sprat weight.

During feeding, the experimental fish were hand fed (ad libitum) to satiation with partially frozen sprats and, as long as the fish quickly swallow the whole sprats, this helps to minimize loss of the marker in the surrounding water. Once the semi-frozen sprat is in the stomach, digestion begins with secretion of acidic gastric juices; the marker mixes well with the sprat tissue to form radio-opaque chyme.

The estimation of maximum stomach capacity using the linear relationship suggested by Seyhan (1994, unpub. observ.) based on distension seems to underestimate the satiation amounts for whiting in this study. Several whiting exceeded the predicted maximum by up to 60%. This may suggest individual variation in stomach size or perhaps an allometric rather than isometric relation between volume and fish weight. Jobling (1981) reviewed evidence that satiation amount increases with body weight raised to a power (0.7-0.8), indicating that smaller fish eat relatively more (g^{-1} bw) than larger fish.

One of the aims of this study was to examine the previous workers (Seyhan, 1994, unpub. observ.; Seyhan *et al.*, 1998) that gastric emptying time remained constant as meal size increased, since the work of Bromley (1988) contradicts this. Both Bromley's fish, and those in the present study, ate much larger voluntary meals than those Seyhan's fish and in both cases gastric emptying time was prolonged well beyond that reported by Seyhan *et al.*, (1998). Small meals were processed at the rate following the equation 1 as predicted by Seyhan (1994, unpub. observ.). His finding that gastric emptying rate (GER) is directly proportional to meal size, thereby maintaining constant GET, may only apply to small meals ($<3\%$ bw) for whiting.

In conclusions, the use of a disperse type radio opaque marker (Barium Sulphate) was found suitable to investigate the gastric emptying time in whiting. The result also shown that the pattern of feed intakes by various sizes of fish denoted an allometric relationship between stomach volume and the fish size. The linear term of gastric emptying process may only suitable to describe the gastric emptying of fish ingesting the small meal ($< 3\%$ bw).

Acknowledgments

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References

Bromley, P. J., 1988. Gastric digestion and evacuation in whiting, *Merlangius merlangus* (L.). J. Fish Biol. , 33: 331-338. dos Santos, J. and M. Jobling, 1990. Aspects of gastric evacuation in the Atlantic cod (*Gadus morhua* L.). ICES. C. M. 1990/G: 69, pp: 17.

dos Santos, J. and M. Jobling, 1990. Aspects of gastric evacuation in the Atlantic cod (*Gadus morhua* L.). ICES. C. M. 1990/g : 69, pp: 17.

dos Santos, J. and M. Jobling, 1991. Gastric emptying in cod, *Gadus morhua* L. emptying and retention of indigestible solids. J. Fish Biology , 38: 187-197.

Hossain, M. A. R., g. C. Haylor and M. C. M. Beveridge, 1998. An evaluation of radiography in studies of gastric evacuation in African catfish fingerlings. Aquaculture International, 5: 379-385.

Jobling, M., 1981. Mathematical models of gastric emptying and estimation of daily rates of food consumption for fish. J. Fish Biology, 19: 245 - 257.

Jobling, M., A. M. Arnesen, B. M. Baardvik, J. S. Christiansen and E. H. Jorgensen, 1995a. Monitoring feeding behaviour and food intake: Methods and applications. Aquaculture Nutrition, 1: 131-143.

Jobling, M., A. M. Arnesen, B. M. Baardvik, J. S. Christiansen and E. H. Jorgensen, 1995b. Monitoring voluntary feed intake under practical conditions, methods and applications. J. Appl. Ichthyol., 11: 248-262.

Jorgensen, E. and M. Jobling, 1988. Use of radiographic methods in feeding studies: a cautionary note. J. Fish Biology, 32: 487-488.

Seyhan, K., 1994. Gastric emptying, food consumption and ecological impact of whiting, *Merlangius merlangus* (L.) in Irish Sea marine ecosystem. Ph. D. Thesis, University of Wales Bangor.

Seyhan, K., D. J. Grove, J. King, 1998. Feeding behaviour of whiting, *Merlangius merlangus* L. in captivity. Fisheries Res., 34: 39-45.

Sims, D. W., S. J. Davies and Q. Bone, 1996. Gastric emptying rate and return of appetite in lesser spotted dogfish, *Scyliorhinus canicula* (Chondrichthyes: Elasmobranchii). J. Marine Biol. Assoc. of the United Kingdom, 76: 479-491.

Talbot, C. and P. J. Higgins, 1983. A radiographic method for feeding studies on fish using metallic iron powder as a marker. J. Fish Biology, 23: 211-220.